

Studies Regarding the Colonization Capacity of Soils with Permanent Nitrogen Fixating Bacteria, Located on Different Altitudinal Levels

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Abstract

The determination of the colonization capacity with permanent nitrogen fixating bacteria has been achieved indirectly through the method of using soil extracts, taken from the rhizosphere of leguminous species existing in the 4 types of permanent grasslands, located on different altitudinal levels (90m, 330m, 900m, 1800m). Treatments with soil extracts taken have been made at three species of legumes (*Lotus corniculatus*, *Trifolium repens*, *Trifolium pratense*), seeded on a sown perlite layer and grown in the growth chamber. Between the total amount of nodosities formed and the altitude of grasslands there is a negative correlation. At treatments with extracts taken from grasslands situated between 90-330m, there has been observed the highest number of nodosities formed on roots of tested leguminous species. .

Keywords: altitude, colonization capacity, grassland, legumes, nodosities

1. Introduction

Permanent grasslands are situated mostly in areas with higher altitudes where climatic conditions are less favourable, both for leguminous plant growth and for multiplying nitrogen fixing bacteria. In these grasslands, the nitrogen fixing capacity is lower as compared to grasslands in lower altitude areas [1-5].

This paper aims at highlighting the ability to colonize soil with nitrogen fixing bacteria in permanent grassland, situated at different altitudes, indirectly, through the study of nodosities formation in some species of legumes grown "ex situ".

2. Materials and methods

The determination of the colonization capacity with permanent nitrogen fixating bacteria has been achieved indirectly through the method of using soil extracts, taken from the rhizosphere of leguminous species existing in the 4 types of permanent grasslands, located on different altitudinal levels: 90 m (Jebel- Timiș), 330 m (Caransebeș), 900 m (Poiana Mărului), 1800 m (Blana- Bucegi).

From the soil samples taken from each type of grassland, 100 g of soil were extracted and introduced in an amount of 100 ml of double distilled water. The soil solution + double distilled water have been filtered with filter paper. Seeds from three species of legumes (*Lotus corniculatus*, *Trifolium repens*, *Trifolium pratense*) were sterilized before sowing with ethanol 70%, for 3 minutes. After sterilization,

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seeds were sown with 20 seeds, in 3 repetitions, in containers with 10 cm diameter perlite and 6 cm height.



Photo 1 – Containers with perlite sown with leguminous species

Containers with perlite sown with three species of legumes have been kept up to germination in the laboratory, then were placed in the growth chamber, where environmental conditions were controlled (night/day temperature of +15-20°C, humidity of 70%, photoperiod of 16h).

The soil extract from each type of lawn was applied immediately after sowing and at intervals of 7-10 days, with a quantity of 50 ml /container. The number of nodosities/plant, indicating the colonisation capacity of soils in the grassland studied with nitrogen fixing bacteria, has been determined, in all variants, after 45 days from containers being introduced into the growth chamber.

3. Results and discussion

The presence of legume species in permanent grassland floristic structure shows that within these natural ecosystems there is a kind of activity, more or less intense of the atmospheric nitrogen. The amount of nitrogen fixed in the permanent grasslands is conditioned both by the proportion of leguminous species in the vegetal cover and by the

colonization capacity of soils with nitrogen fixing bacteria.

Following the studies carried out, it has been observed that at all the three leguminous species under study the treatment with double distilled water has not lead to formation of nodosities. However, at the treated variants with soil extracts, nodosities were observed at all leguminous species and their number differed according to the altitude of the grasslands.

Treatments with soil extract, taken from the rizosphere of leguminous species in grasslands have positively influenced the formation of nodosities at the specie *L. corniculatus* (Figure 1).

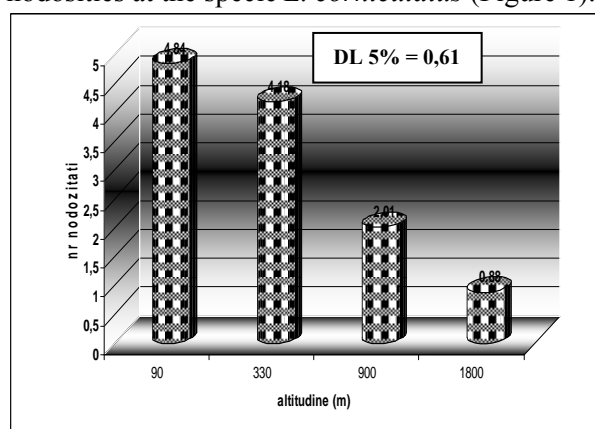


Figure 1- The influence of treatments with soil extracts, taken from grasslands located at different altitudes, on the number of *Lotus corniculatus* nodosities.

Although nodosities have been formed at all treated variants, the highest number has been recorded at variants with soil samples taken from grasslands in low altitudes (90 and 330m), where 4.84 nodosities/ plant were determined and 4.18 nodosities/ plant. In the case of alpine grasslands (900m) and subalpine (1800m) the colonisation capacity with nitrogen fixing bacteria decreases, being also shown by the low number of nodosities formed at *L. corniculatus*: 2.01 and 0.88.

The studies that have examined *Trifolium repens* as testing specie have shown a higher increase in the nodulation capacity under the treatment with soil extracts taken from grasslands located at altitudes of 330-900m, where there have been recorded, on average, 3.84 and 2.49 nodosities/ plant. At the altitude of 1800m the number of nodosities has been only of 0.48/plant (Figure 2).

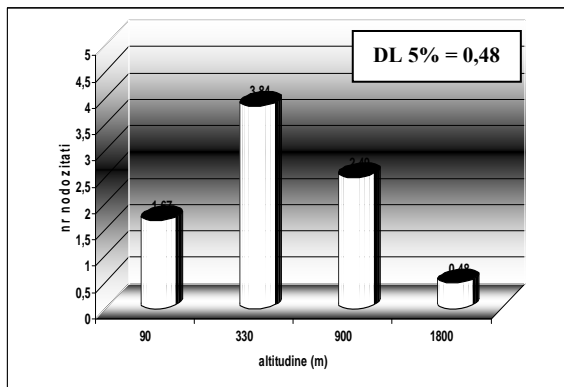


Figure 2. The influence of soil treatments with extracts from grasslands altitudes away over the number of nodosities at *T. repens*

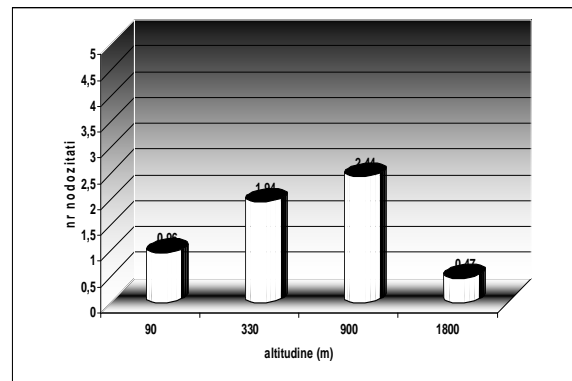


Figure 3. The influence of soil treatments with extracts from grasslands altitudes away over the number of nodosities at *T. pratense*

In what regards the test of nodosities formation at the specie *Trifolium pratense* there was observed a higher increase at variants with extracts taken from the grassland soils located at altitudes between 330-900 m (Figure 3).

The shape of the regression curves shows that between the altitude where grasslands are located and the number of nodosities formed at the testes leguminous species, with soil extracts taken from these grasslands, there is a reverse correlation, more obvious at the specie *L. corniculatus* (Figure 4).

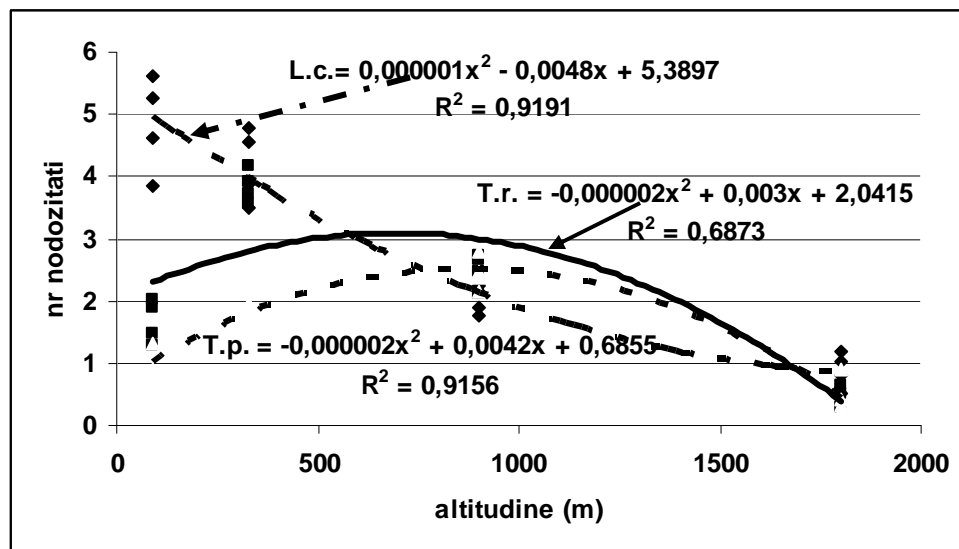


Figure 4. The correlation between altitude and the number of nodosities species of legumes grown in situ

The colonization capacity with permanent nitrogen fixating bacteria of soils in the studied permanent grasslands, determined by relating to the total number of nodosities/ plant formed at leguminous species treated with soil extracts, demonstrates that grasslands in the plains and hills areas (altitude between 90-900 m), where the

highest number of nodosities was recorded (6.94-9.97), would also have the highest capacity of colonizing with nitrogen fixing bacteria. In the subalpine grasslands (over1800m) the lowest number of nodosities was recorded (1.83/plant) and consequently the lowest colonisation capacity (Table 1).

Table 1. The colonization capacity of soils with permanent nitrogen fixating bacteria, situated on different altitudinal levels

Altitude	The total number of nodosities formed at the studied leguminous species	The colonization capacity (%)
90m	7.47	100.0
330m	9.97	133.5
900m	6.94	92.4
1800m	1.83	24.5

4. Conclusions

The colonization capacity with nitrogen fixing bacteria of the permanent grasslands can be indirectly highlighted, such as the method of determining the number of nodosities formed at leguminous species cultivated "ex situ", treated with soil extracts taken from the rizosphere of the existing leguminous species "in situ" in the vegetal cover of these grasslands.

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